UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

FEB 23 1994

C10794

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 4-CPA (4-Chlorophenoxyacetic acid) - Subchronic Dietary Admix Toxicity Study in Dogs (§82-1B)

DP Barcode: D197898 **Case:** 802264
Submission: S455750 **PC Code:** 019401
Identification No.: 019401-008906
MRID Nos.: 13-Week Study = 429683-01
Escalation Dose = 430453-01
Action: 627 Core Data

FROM: Alan C. Levy, Ph.D., Toxicologist Alan C. Levy
Review Section IV, Toxicology Branch II 2-17-94
Health Effects Division (7509C)

TO: Kathryn Davis/Thomas Luminello, Jr., PM 52
Special Review and Reregistration Division (7508W)

THRU: Jess Rowland, M.S., Acting Section Head Jess Rowland 2/17/94.
Review Section IV, Toxicology Branch II
Health Effects Division (7509C)

and

Marcia van Gemert, Ph.D., Branch Chief
Toxicology Branch II
Health Effects Division (7509C) M. van Gemert 2/17/94

REQUEST: Review a 13-week dietary admix dog study with 4-CPA.

Registrant: Hunt-Wesson, Inc.

EXECUTIVE SUMMARY:

In a subchronic toxicity study, 4-chlorophenoxyacetic acid (4-CPA) was administered by dietary admix to Ridglan Farms beagle dogs (4/sex/dose) at doses of 0, 20, 100 and 500 ppm (mean mg/kg body weight/day: males = 0, 0.804, 3.286 and 18.582; females = 0, 0.780, 4.047 and 17.410) for 13 weeks. The following parameters were examined: body weights, body weight gains, food consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, organ weights and microscopic pathology. **[MRID 429683-01]**

The only test article related effect appeared at 500 ppm in males and females where there was a 22-23% decrease in body weight gain as well as a decrease in food consumption during approximately the first 4 weeks of the study. **The NOEL is 100 ppm (3.286 mg/kg for males and 4.047 mg/kg for females). The LOEL is 500 ppm (18.582 mg/kg for males and 17.410 mg/kg for females).**

Core Classification is **Minimum.** This study satisfies the data requirement (§82-1B) for a 13-week subchronic toxicity study in dogs.

Per the attached **Toxicology Profile**, all Toxicology Guidelines have been satisfied for this low-volume minor-use chemical.

TOXICOLOGY PROFILE FOR 4-CHLOROPHENOXYACETIC ACID (4-CPA)

§ No.	TYPE OF STUDY	REQUIRED	STATUS	MRID No.	TOX CATEGORY NOEL/LOEL
81-1	Acute oral - rat	Yes	Satisfied	418370-01	III
81-2	Acute dermal - rabbit	Yes	Satisfied	425266-01	III
81-3	Acute inhalation - rat	Yes	Satisfied	429682-01	IV
81-4	Prim. eye irrit. - rabbit	Yes	Satisfied	423391-01	I
81-5	Primary dermal - rabbit	Yes	Satisfied	423068-01	IV
81-6	Dermal sensitiz. - g. pig	Yes	Satisfied	423391-02	Non Sens.
82-1A	Subchronic - rat	Yes	Satisfied	429025-01	NOEL=2000 LOEL=8000 ppm
82-1B	Subchronic - dog	Yes	Satisfied	429683-01	NOEL=100 LOEL=500 ppm
83-3A	Developmental - rat	Yes	Satisfied	423226-02	Mat NOEL= 150 mg/kg Mat LOEL= 300 mg/kg Dev NOEL= 150 mg/kg Dev LOEL= 300 mg/kg
84-2	Muta. - Ames	Yes	Satisfied	418370-02	Non-Muta
84-2	Muta. - Micronucleus	Yes	Satisfied	418370-03	Non-Muta
84-2	Muta. - Mouse lymphoma	Yes	Satisfied	418370-04	Non-Muta

Reviewed by: Alan C. Levy, Ph.D. Alan C. Levy 2-17-94
Section IV, Tox. Branch II

Secondary reviewer: Jess Rowland, M.S. Jess Rowland 2-17-94
Section IV, Tox. Branch II

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Toxicity Study - Dogs (§82-1B)

TEST MATERIAL: 4-CPA: 4-Chlorophenoxyacetic acid

SYNONYMS: none

PC Code: 019401 **MRID Nos:** 13-Week Study = 429683-01
Escalation Dose Study = 430453-01

STUDY NUMBERS: 13-Week = HWI 6341-108; Escalation Dose = HWI 6341-106

SPONSOR: Hunt-Wesson, Inc., Fullerton, CA

TESTING FACILITY: Hazleton Wisconsin, Inc.

TITLE OF REPORT: 13-Week Dietary Toxicity Study with 4-Chlorophenoxyacetic Acid in Dogs
Dietary Escalating Dose Tolerance Study with 4-Chlorophenoxyacetic Acid in Dogs

AUTHOR: Susan M. Henwood

REPORT ISSUED: 13-Week = September 30, 1993
Escalating Dose = September 11, 1992

EXECUTIVE SUMMARY:

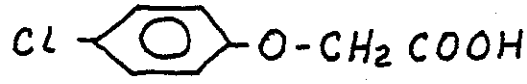
In a subchronic toxicity study, 4-chlorophenoxyacetic acid (4-CPA) was administered by dietary admix to Ridglan Farms beagle dogs (4/sex/dose) at doses of 0, 20, 100 and 500 ppm (mean mg/kg body weight/day: males = 0, 0.804, 3.286 and 18.582; females = 0, 0.780, 4.047 and 17.410) for 13 weeks. The following parameters were examined: body weights, body weight gains, food consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, organ weights and microscopic pathology. **[MRID 429683-01]**

The only test article related effect appeared at 500 ppm in males and females where there was a 22-23% decrease in body weight gain as well as a decrease in food consumption during approximately the first 4 weeks of the study. **The NOEL is 100 ppm (3.286 mg/kg for males and 4.047 mg/kg for females). The LOEL is 500 ppm (18.582 mg/kg for males and 17.410 mg/kg for females).**

Core Classification is **Minimum.** This study satisfies the data requirement (§82-1B) for a 13-week subchronic toxicity study in dogs.

I. TEST ARTICLE

Name: 4-CPA; 4-Chlorophenoxyacetic acid
Formula:



II. ESCALATING DOSE TOLERANCE STUDY (MRID No. 430453-01)

The purpose of this study was to assess palatability of the test material and to establish dose levels for the 4- and 13-week studies.

A. Test Article Description

Purity: 99%
HWI Sample No. = 0020A3 (11/20/91)
Physical Property: white powder

B. Test Article Homogeneity and Stability

Table 1

TEST ARTICLE HOMOGENEITY AND STABILITY IN AN ESCALATING DOSE DOG STUDY WITH 4-CPA

Sample	40 ppm	1600 ppm
top	37.0(93), 37.5(94)	1550(97), 1550(97)
left	37.1(93), 37.0(93)	1550(97), 1550(97)
right	37.3(93), 37.0(93)	1560(98), 1560(98)
bottom	37.1(93), 36.6(92)	1530(96), 1570(98)
Day 0	37.1(93), 36.6(92)	1570(98), 1580(99)
10 days (room temperat)	38.3(96), 38.4(96)	1560(98), 1530(96)

() = % of theoretical
Data extracted from Report Tables 1 and 2, pages 20 and 21.

Homogeneity and stability data were within acceptable limits.

C. Study Design

There were 2 male and 2 female controls. Two males and 2 females were in the treated group and received concentrations (ppm) of test article (dietary admix) as follows:

Day 1 = 40, 4 = 200, 7 = 400, 10 = 800, 13 = 1,600, 16 = 3,200 and days 19 through 28 = 1,600

The test article diets were presented to the dogs for a period of 3 hours each dosing day with basal diets presented for 3 hours/day for 2 days (see schedule above).

D. Animals

Male and female beagles were received from Harlan Sprague Dawley, Inc. The dogs were individually housed (stainless steel cages) in rooms set to maintain temperature and humidity at 19-26°C and 50±20%, respectively. There was a 12 hour light/dark cycle. Food was available ad libitum during the 1st week of acclimation (total acclimation period was 22 days) followed by 2 weeks (prior to treatment) of 3 hours/day and continuing the 3 hours/day for the treatment period. Water was available ad libitum.

E. Observations

These were made A.M. and P.M. daily for mortality and moribundity. Cageside observations were performed prior to dosing and hourly (for 4 hours) after presentation of food. In addition, the animals were observed A.M. on non-dosing days.

There was no mortality.

Of the 4 treated dogs, liquid feces were reported on days 1 (1/4 dogs), 4 (3), 5 (2), 6 (2), 7 (2), 8 (2), 9 (2) and 10 (2). This observation was not noted after day 10. One control dog (out of 4) had liquid feces on days 1, 3, 4, 15, 20 and 29.

F. Body Weights

These were recorded weekly for 3 weeks pre-treatment, daily during treatment and the day of necropsy. Table 2.

Over the 28 day dosing period, control males gained 1.6 and 1.8 kg compared with treated dogs which gained 1.1 and 1.2 kg. During continuous dosing of 1,600 ppm (days 19-28), control males gained 0.3 and 0.6 kg; whereas, treated animals each lost 0.2 kg.

For females, over the 28 days, controls each gained 2.0 kg compared with 0.6 and 0.3 kg for the treated dogs. While receiving 1,600 ppm continuously (days 19-28), controls gained 0.8 and 0.7 kg with treated females losing 0.5 and 0.7 kg.

Table 2

SELECTED INDIVIDUAL BODY WEIGHTS DURING AN ESCALATING DOSE TOLERANCE STUDY IN DOGS WITH 4-CPA

Dog			40a	200	400	800	1600	3200	1600		
		-7b	1	4	7	10	13	16	19	28	-7 to 28
49M	C	6.60	6.90	7.10	7.30	7.50	7.67	7.90	7.90	8.20	1.60
53M	C	6.20	6.40	6.60	6.80	7.00	7.30	7.40	7.40	8.00	1.80
51M	T	6.90	7.00	7.30	7.40	7.60	8.00	8.20	8.20	8.00	1.10
52M	T	6.60	6.90	7.10	7.30	7.50	7.80	7.90	8.00	7.80	1.20
54F	C	7.10	7.20	7.40	7.50	7.80	8.10	8.30	8.30	9.10	2.00
55F	C	6.60	7.00	7.10	7.30	7.50	7.80	7.80	7.90	8.60	2.00
56F	T	6.40	6.80	6.80	6.90	7.10	7.40	7.60	7.50	7.00	0.60
57F	T	7.10	7.40	7.60	7.60	7.80	8.10	8.10	8.10	7.40	0.30

C = Control; T = Treated
a = ppm; b = study day
values = kg body weight
Data extracted from Report Table 4, pages 23-28.

G. Food Consumption

This was recorded each day starting 1 week prior to test article administration.

Treated males and females ate about the same amount of food as did controls through dosing day 13 when they received 1,600 ppm. When the treated group was given 3,200 ppm, the control males consumed 210 and 298 g compared with 60 and 132 g for treated dogs; for females, controls ate 246 and 273 g compared with 83 and 89 g for treated animals. During continuous 1,600 ppm treatment (days 19-28), treated males ate about 65-80% of control consumption and treated females, about 30-60%.

H. Necropsy

The day after the last dosing day, fasted dogs were weighed, anesthetized with sodium pentobarbital, exsanguinated and necropsied. Tissues with gross lesions were fixed in 10% phosphate-buffered formalin.

There were no test article related macroscopic findings.

I. Discussion

The primary observation during the 28 day study was liquid feces mostly in 2/4 treated animals during the first 10 days. One control showed this on days 1, 3, 4, 15, 20 and 29. As this finding was not reported when treated dogs received 1,600 or 3,200 ppm, it appears unlikely that it was test article related.

Body weights were reduced at 1,600 and 3,200 ppm along with a relatively severe decrease in food consumption at 3,200 ppm. During the 10 days of dosing with 1,600 ppm, all 4 treated dogs lost weight (all 4 controls gained). The Report Author suggested that the 3,200 ppm diet was not palatable.

J. Conclusion

The approximate Maximum Tolerated Dose (MTD) was determined to be 1,600 ppm based on decreased body weight and food consumption.

Based upon these findings, the doses chosen for a 4-week study (HWI 6341-107) were 200, 400, 800 and 1,200 ppm.

III. 13-WEEK STUDY (MRID No. 429683-01)

A. Statistical Analyses

LEVENE'S TEST - to test for variance homogeneity

ANALYSIS OF VARIANCE (ANOVA) - on homogeneous or transformed data

DUNNETT'S TEST - if ANOVA was significant, for pairwise comparison between all groups

ONE-WAY ANOVA (if applicable) - initial body weights, body weight gains, food consumption, clinical chemistry, applicable hematology, applicable urine parameters, organ weights, organ-to-body weight percentages and organ-to-brain weight ratios

ONE-WAY ANOVA WITH PLANNED COMPARISONS - body weights (with initial weight as covariate); if significant, Dunnett's test was used

B. Regulatory Compliance

A Good Laboratory Practice Compliance statement, Quality Assurance statement and a list of Quality Assurance inspections were included in the Report.

The Registrant applied the criteria of 40 CFR 158.34, flagging studies for potential adverse effects, to the results of this study and determined that the study neither met nor exceeded any of the applicable criteria.

A signed statement of no confidentiality claim was provided.

C. Test Article Description

Physical Property: white powder
Material Received: shipments of 5/20/92 and 12/9/92 (Hazleton Sample Nos. 00200A4 and 0020A6, respectively) - The 5/20/92 sample was used for homogeneity and stability analyses only.
Purity: "at least 99.8%"

D. Test Article Homogeneity, Stability and Analyses

Table 3

TEST ARTICLE HOMOGENEITY IN A 13-WEEK DOG STUDY WITH 4-CPA

Sample Location	Prestudy Mix		Week 2 Mix	
	20 ppm	500 ppm	20 ppm	500 ppm
top	94%	95%	96%	94%
left	91%	94%	93%	95%
right	89%	93%	92%	91%
bottom	92%	93%	98%	92%

NOTE: All % of theoretical are means of 2 values.
Data extracted from Report Tables 1 and 2, pages 41 and 42.

Table 4

TEST ARTICLE STABILITY IN A 13-WEEK DOG STUDY WITH 4-CPA

Storage Conditions	Prestudy Mix		Week 2 Mix		Week 9 Mix	
	20 ppm	500 ppm	20 ppm	500 ppm	20 ppm	500 ppm
Day 0/Initial.....	96%	97%	95%	98%	90%	96%
2 week/room temp....	102%	24%	92%	95%	-	-
2 week/room temp (a)	-	94%	-	-	-	-
2 week/frozen.....	101%	15%	94%	95%	-	-
2 week/frozen (a)...	-	96%	-	-	-	-
7 week/frozen.....	92%	95%	-	-	109%	108%

(a) = reanalysis of extract
NOTE: All %s of theoretical are means of 2 values.
- = not analyzed
Data extracted from Report Tables 3-5, pages 43-45.

Table 5

SELECTED TEST ARTICLE ANALYSIS DATA IN A 13-WEEK DOG STUDY WITH 4-CPA

ppm	Week										
	1	4	5	6	7	8	9	10	11	12	13
20	92%	95	93	-	-	93	-	-	103	-	-
100	97	92	-	98	-	-	107	-	-	101	-
500	100	96	-	-	94	-	-	106	-	-	107

NOTE: All % of the theoretical are means of 2 values.
Data extracted from Report Table 6, pages 46 and 47.

Homogeneity, stability and analysis values were considered to be within acceptable limits.

E. Dose Selection (Report page 13)

In a 4-week range-finding study (HWI-6341-107) with 2 beagles/sex/group, the dogs were fed a dietary admix of 0, 200, 400, 800 and 1,200 ppm of 4-CPA for about 3 hours/day. A decrease in body weights and food consumption at 800 and 1,200 ppm was attributed to decreased palatability. There were increases in urea nitrogen, creatinine and cholesterol with a lower absolute lymphocyte count. These findings, plus, "Changes in absolute and relative organ weights ..." were attributed to, "... decreased nutritional status." There was no identification of specific organ toxicity.

Based on these results, doses selected for the 13-week study were 0, 20, 100 and 500 ppm.

F. Diet Preparation

Concentrations were based on the test article as supplied by the Sponsor to the Testing Facility. A premix was prepared for the 100 and 500 ppm diets. For the 20 ppm concentration, a specific amount of 500 ppm diet was added to basal diet. Appropriate concentrations were prepared each week and stored at room temperature.

G. Animals

Male and female beagles were received from Ridglan Farms, Mt. Horeb, WI. They were about 4-6 months of age when treatment began, with males weighing 5.8-8.0 kg and females, 5.8-7.7 kg. The dogs were individually housed and acclimated to room conditions for 20 days. Room temperature and humidity were set for 19-26°C and 50±20%, respectively. There was a 12-hour light/dark cycle. Two weeks prior to the start of treatment, the dogs were acclimated to the 3-hour/day feeding schedule. Water was available ad libitum. The animals were stratified by body weight and assigned 4/sex/group by computer-generated randomization.

H. Mortality and Clinical Signs

The dogs were observed A.M. and P.M. daily. There were no deaths.

Red-colored feces was reported in three 100 ppm males a total of 6 days, one 500 ppm male on one day, one control female on one day and one 500 ppm female on 2 days.

Vomitus was observed during the study. Table 6.

Table 6

THE APPEARANCE OF VOMITUS IN A 13-WEEK DOG STUDY WITH 4-CPA

Vomitus	Males (ppm)				Females (ppm)			
	0	20	100	500	0	20	100	500
cloudy	2/6	2/3	4/6	4/9	3/11	3/8	3/3	4/23
clear	1/1	0	2/3	0	0	0	0	1/1
containing food ..	0	1/1	2/2	2/4	2/2	1/1	0	3/6

#/# = number of dogs with observation out of 4 dogs/total number of days observation was made

Data extracted from Report Tables 7, 8 and Appendix B, pages 48-50 and 160-178.

The animals were sacrificed on study days 95, 96 or 97. There were at least 376 dog-days/group (4 dogs x 94 days). The 500 ppm females appeared to have a greater incidence of cloudy vomitus (23 days) than did the controls (11 days). The four 500 ppm females had cloudy vomitus on 11, 3, 2 and 7 days versus 0, 5, 1 and 5 days for controls. As the highest incidence for any dog was about 12% of the days, it is questionable that the number of dogs and the incidence of the vomitus observation was a result of test article administration.

I. Body Weights

These were recorded at weeks -2, -1 and weekly until study termination (including the day of necropsy).

Table 7

GROUP MEAN BODY WEIGHTS AND WEIGHT GAINS IN A 13-WEEK DOG STUDY WITH 4-CPA

Week	Males (ppm)				Females (ppm)			
	0	20	100	500	0	20	100	500
BODY WT								
-1	6900	6975	6700	6875	6625	6350	6425	6575
1	7000	7050	6850	7125	6775	6500	6575	6600
2	7400	7350	7150	7275	7150	6800	6975	6825
3	7650	7675	7425	7575	7425	7050	7225	7100
4	8025	8000	7800	7750	7600	7375	7450	7325
6	8675	8650	8250	8350	8000	7775	7875	7775
8	9075	9050	8750	8825	8400	8225	8325	8250
10	9575	9650	9250	9400	8725	8675	8625	8600
12	9875	9925	9550	9625	9125	8925	9025	9000
14	10275	10300	9775	10150	9300	9150	9175	9150
BW GAIN								
-1-1	100	75	150	250	150	150	150	25
-1-4	1125	1025	1100	875	975	1025	1025	750
4-8	1050	1050	950	1075	800	850	875	925
8-14	1200	1250	1025	1325	900	925	850	900
-1-14	3375	3325	3075	3275	2675	2800	2750	2575

BODY WT = body weight; BW GAIN = body weight gain; all values = g
NOTE: Body weight gains calculated by Reviewer as Report Tables 11 and 12, pages 57-60, presented only cumulative values.
Data extracted from Report Tables 9 and 10, pages 51-56.

Group mean body weights were similar for males and females throughout the study (no statistically significant differences). Over the 13 weeks of the study, group mean body weight gains (weeks -1 to 14) for treated dogs were similar to respective controls. Regarding gains during the

early period (weeks -1 to 4) of treatment, there appeared to be a lesser group mean gain at 500 ppm for both males and females (see Table 7).

J. Food Consumption

This was measured weekly starting one week prior to treatment.

The group mean amount of food eaten/week by treated animals was similar to respective controls except during approximately the first 4 weeks of the study where there appeared to be a decrease in the 500 ppm animals of both sexes and the 100 ppm males. In the 100 ppm males, weeks 9 and 12 were significantly ($p<0.05$) lower than the control value. The Report indicated that this reduced food intake may have been due to a palatability problem.

K. Test Article Consumption (Table 8)

Table 8

GROUP MEAN TEST ARTICLE CONSUMPTION IN A 13-WEEK DOG STUDY WITH 4-CPA

	Males (ppm)			Females (ppm)		
	20	100	500	20	100	500
Mean (mg/kg/day)	0.804	3.286	18.582	0.780	4.047	17.410

NOTE: mg/kg/day were calculated from the ppm diet concentration, body weights and food consumption weekly over the 13-week study
Data extracted from Report Tables 15 and 16, pages 67-70.

L. Ophthalmic Examinations

Ophthalmoscopy was performed on all dogs prior to treatment and before sacrifice utilizing an indirect ophthalmoscope.

There were no findings attributed to the administration of 4-CPA.

M. Clinical Pathology

Dogs were fasted overnight and blood for hematology and clinical chemistry was collected from the jugular vein prior to treatment as well as during weeks 5 and 14. Urine was collected overnight (about 16 hours).

HEMATOLOGY

The following parameters were examined:

Erythrocyte count*
Hemoglobin*
Hematocrit*
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Activated partial thromboplastin time*
Reticulocyte count smear (made, not examined)
Leukocyte count*
Leukocyte differential*
Blood cell morphology
Platelet count*
Prothrombin time*

*= EPA Guideline Requirement

A questionable (not statistically significant) increase in hemoglobin and hematocrit group mean values was noted.

Table 9

SELECTED GROUP MEAN HEMATOLOGY VALUES IN A 13-WEEK DOG STUDY WITH 4-CPA

Parameter	Males (ppm)				Females (ppm)			
	0	20	100	500	0	20	100	500
WEEK -2								
Erythrocyte-mean	6.62	5.95	6.55	6.62	6.28	6.28	6.12	6.50
-S.D.	.506	.597	.526	.150	.359	0.50	.377	.294
Hemoglobin -mean	14.1	13.2	13.9	14.2	13.9	13.8	13.6	14.4
-S.D.	.75	.79	1.29	.44	.99	.54	.72	.58
Hematocrit -mean	41.3	38.2	41.0	41.9	40.5	40.1	39.8	41.6
-S.D.	2.54	2.59	3.40	1.20	2.49	1.38	1.85	1.79
WEEK 5								
Erythrocyte-mean	6.28	5.82	6.38	6.82	6.32	6.32	6.68	6.72
-S.D.	.236	.377	.629	.411	.222	.386	.866	.618
Hemoglobin -mean	13.8	13.2	14.0	14.9	14.2	14.2	15.3	15.2
-S.D.	.81	.70	1.24	.47	.45	.79	1.52	1.36
Hematocrit -mean	39.1	37.4	40.0	42.8	41.0	40.8	43.7	43.1
-S.D.	1.99	1.65	3.76	1.39	1.16	2.09	4.63	3.58
WEEK 14								
Erythrocyte-mean	6.62	6.35	6.95	7.05	7.00	6.68	6.88	7.58
-S.D.	.450	.300	.827	.191	.183	.492	.450	.435
Hemoglobin -mean	14.9	14.7	15.7	15.9	16.1	15.6	16.2	17.3
-S.D.	.71	.40	1.76	.74	.82	1.22	1.18	.94
Hematocrit -mean	42.2	41.6	44.3	45.3	45.9	44.3	45.6	49.3
-S.D.	1.90	.80	4.87	2.24	1.63	3.52	3.35	2.30

Erythrocyte = 10^6/mL; Hemoglobin = G/DL; Hematocrit =%
Data extracted from Report Tables 18-23, pages 72-83.

CLINICAL CHEMISTRY

The following parameters were examined:

Glucose*	Alanine aminotransferase*
Urea nitrogen*	Alkaline phosphatase
Creatinine*	Creatine kinase
Total protein*	Calcium*
Albumin*	Inorganic phosphorus*
Globulin	Sodium*
Total bilirubin*	Potassium*
Cholesterol	Chloride*
Aspartate aminotransferase*	

* = EPA Guideline Requirements

There were no differences between treated and control groups which were considered to be related to test article administration.

URINALYSIS (not required by EPA Guidelines)

The following parameters were examined:

Specific gravity	Blood
pH	Urobilinogen
Protein	Volume (about 16 hours)
Glucose	Microscopic examination of sediment
Ketones	Appearance
Bilirubin	

There were no apparent test article associated differences regarding any of the parameters.

N. Anatomical Pathology

After 13 weeks of test article administration, the dogs were fasted overnight, weighed, anesthetized (sodium pentobarbital), exsanguinated and necropsied. The following organs were weighed: brain, kidneys, liver, ovaries, testes and thyroids/parathyroids. Organ weights were expressed as absolute, organ-to-body weight percentages and organ-to-brain weight ratios. The following tissues were preserved in 10% phosphate-buffered formalin and examined microscopically for all animals (organs with an "x" were weighed:

DIGESTIVE	RESPIRATORY	UROGENITAL
Salivary gland*	Trachea*	xKidneys*
Esophagus*	Lung*	Urinary bladder*
Stomach*		xTestes*
Duodenum*	CARDIOVASC/HEMAT	xOvaries
Jejunum*	Aorta*	Cervix
Ileum*	Heart*	Uterus*
Cecum*	Bone marrow*	Epididymides
Colon*	Lymph nodes*	Prostate
Rectum*	Spleen*	Vagina
xLiver*	Thymus*	
Pancreas*		
Gallbladder*		

NEUROLOGIC	GLANDULAR	OTHER
xBrain*	Adrenals*	Bone*
Peripheral nerve*	Mammary gland*	Skeletal muscle*
Spinal cord (3 levels)*	xParathyroid*	Skin
Pituitary*	xThyroid*	Gross lesions and masses*
Eyes (with optic n.)*		

* = EPA Guideline Requirements

MACROSCOPIC

There were no macroscopic findings which appeared to be related to test article administration.

ORGAN WEIGHTS

The ovary appeared to be the only organ which may have been effected by test article administration. Tables 10 and 11.

Table 10

A SUMMARY OF GROUP MEAN OVARIAN WEIGHTS IN A 13-WEEK DOG STUDY WITH 4-CPA

ppm	Fin BW (g)	Absolute (g)			Organ-to-BW (%)			Organ-to-brain ratio		
		left	right	comb	left	right	comb	left	right	comb
0	9175	.300	.295	.595	.33a	.33a	.66a	.39a	.39a	.78a
20	9150	.466*	.430	.896	.52*	.47	.99	.63*	.58	1.21
100	9200	.367	.367	.734	.41	.41	.82	.48	.48	.96
500	9075	.441*	.355	.796	.48*	.39	.87	.57	.46	1.03

a = Organ-to-body weight (%) and organ-to-brain ratio values should be multiplied by 10^{-2}.
Statistical Significance: * = $p<0.05$
Data extracted from Report Tables 36-38, pages 102-110.

Table 11

INDIVIDUAL FEMALE FINAL BODY WEIGHTS AND ABSOLUTE OVARIAN WEIGHTS (g) IN A 13-WEEK DOG STUDY WITH 4-CPA

ppm	1st Dog			2nd Dog			3rd Dog			4th Dog		
	BW	LO	RO	BW	LO	RO	BW	LO	RO	BW	LO	RO
0	9.6	320	261	9.2	308	221	8.1	285	364	9.8	288	334
20	10.4	470	531	10.1	470	436	8.2	484	394	7.9	439	359
100	8.3	462	380	10.8	343	406	7.8	325	387	9.9	337	296
500	8.7	340	313	8.6	451	360	9.8	565	315	9.2	408	433

BW = Body Weight; LO = Left Ovary; RO = Right Ovary
Body weights in kg; ovarian weights in mg
Data extracted from Report Appendix D, pages 283-286.

Statistical occurance was noted only for the left ovary and in the 20 and 500 ppm groups, but not in the 100 ppm dogs. When left and right ovarian weights were combined [by the Reviewer] (Table 10), there appeared to be the suggestion of greater absolute, organ-to-body weight % and organ-to-brain ratio in treated dogs. There was no apparent dose-response effect.

MICROSCOPIC

There were no microscopic findings which differentiated treated from control groups.

O. Discussion

Homogeneity, stability and analysis values were considered to be within acceptable limits.

Mean test article consumption as mg/kg body weight/day throughout the 13-week study for the 20, 100 and 500 ppm groups was as follows: males = 0.804, 3.286 and 18.582; females = 0.780, 4.047 and 17.410.

The only suggestive clinical sign possibly due to 4-CPA administration was an increase in the incidence of vomitus (cloudy and/or containing food) in the 500 ppm dogs, particularly in females.

Group mean body weight gains were lower in males and females of the 500 ppm group during the first 4 weeks of the study. In males, there was a 22% reduction in weight gain, and in females, 23%. During about the same time period, the

amount of food consumed by the 500 ppm dogs was less than what was eaten by the controls. The Report indicated that this may have been a palatability problem.

There were no ophthalmic, hematology, clinical chemistry or urinalysis parameters which seemed to be altered by test article administration.

An increase in absolute and organ-to-body weight percent of ovaries was only suggested. There were increases ($p<0.05$) in left ovarian weights at 20 ppm (absolute, relative-to-body weight and relative-to-brain weight) and at 500 ppm (absolute and relative-to-body weight). There were no significant differences at the 100 ppm concentration or for the right ovary (see Tables 10 and 11). Taking into consideration the left-right ovarian weight differences, the individual final body weights, the significant differences for only the left ovary, the relatively small differences from control and the lack of any microscopic findings, it is not considered that the possible ovarian weight differences are of severe toxicological significance.

IV. CONCLUSIONS

In a subchronic toxicity study, 4-chlorophenoxyacetic acid (4-CPA) was administered by dietary admix to Ridglan Farms beagle dogs (4/sex/dose) at doses of 0, 20, 100 and 500 ppm (mean mg/kg body weight/day: males = 0, 0.804, 3.286 and 18.582; females = 0, 0.780, 4.047 and 17.410) for 13 weeks. The following parameters were examined: body weights, body weight gains, food consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, organ weights and microscopic pathology. **[MRID 429683-01]**

The only test article related effect appeared at 500 ppm in males and females where there was a 22-23% decrease in body weight gain as well as a decrease in food consumption during approximately the first 4 weeks of the study. **The NOEL is 100 ppm (3.286 mg/kg for males and 4.047 mg/kg for females). The LOEL is 500 ppm (18.582 mg/kg for males and 17.410 mg/kg for females).**

Core Classification is **Minimum.** This study satisfies the data requirement (§82-1B) for a 13-week subchronic toxicity study in dogs.

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